



Agricultural Research Institute, Pusa.

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OFFICIAL AND RECOMMENDED  
METHODS FOR USE IN CHEMICAL  
LABORATORIES OF THE DEPARTMENTS  
OF AGRICULTURE IN INDIA.

BY

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## PREFACE.

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AT the last Meeting of the Board of Agriculture a Committee of the chemists who were present was appointed to discuss methods of analysis and to decide whether any such should be defined as "Official." The results of the Committee's deliberations were that it is desirable to adopt certain methods as "Official," the use of which will be binding on chemists of the Agricultural Department, and these methods were specified. In other cases certain methods were *recommended* for use. It was also recommended that a synopsis of these methods should be published for use in the Chemical Laboratories of the Department, and the President of the Board sanctioned this publication.

There has been some delay in giving effect to this, but it has been unavoidable. The methods concerned are detailed in this Bulletin. "Official" methods are printed in thick type.

J. WALTER LEATHER,  
*Chairman of the Committee.*

PUSA:  
*The 22nd June 1907.*



OFFICIAL AND RECOMMENDED METHODS FOR  
USE IN CHEMICAL LABORATORIES OF THE DEPARTMENTS  
OF AGRICULTURE IN INDIA.

**SOILS.**

**DEFINITION OF SURFACE SOIL.**—In all ordinary cases, and unless special circumstances interfere, the *surface soil* shall be defined as the first 20 cms.

**DEFINITION OF FINE EARTH.**—After air-drying, the soil shall only be crushed and pulverised with a wooden roller or mallet; it must not be ground in a mortar. The “fine earth” shall be that portion which will pass through a sieve with round holes 1 m.m. diameter.

*Physical Analysis.*

- (i) Sedimentation in beakers is recommended.
- (ii) Depth of water 10 cms.
- (iii) Preliminary treatment of the soil with acid to remove the calcium carbonate was unanimously objected to.
- (iv) Boiling the soil was considered of doubtful utility.
- (v) In all cases the actual or average size of particles must be checked by actual microscopic measurement.
- (vi) The following periods of sedimentation are recommended:—24 hours, 2 hours, 30 minutes, 10 minutes, 75 seconds, sieve 90 meshes to one linear inch.

*Chemical Analysis.*

(1) **Matter soluble in Hydrochloric acid.**—When it is desired to determine constituents which are soluble in concentrated hydrochloric acid the “fine earth” should be first ignited and then heated just at the boiling point on a sand bath for 8 hours in hydrochloric acid of 1.115 specific gravity (26 per cent. HCl).

(2) **AVAILABLE POTASH AND PHOSPHATE.**—Dyer’s method as detailed in *Journal of the Chemical Society*, 1894, page 115:—

“The determinations of solubility in citric acid solution were made as follows:—A weight of air-dried soil corresponding to 200

*A.B.*—Methods which have been adopted as “Official” are printed in thick type.

grams of completely dry soil was placed in a Winchester quart bottle with two litres of distilled water in which were dissolved 20 grams of pure citric acid. (Winchester quarts which had been used for the storage of strong acids were chosen in order to eliminate the possibility of dissolving potash, etc., from the glass. The bottles were rinsed many times, allowed to rest full of water for some days and again rinsed.) The soil was allowed to remain in contact with the 1 per cent. citric acid solution for seven days, being, except on one day, shaken up a great many times each day, whenever, that is to say, the soil had settled down. The bottles rested, however, at night. Altogether the shaking up, which was not violent, but only sufficient to break up the cake of mud which formed each time the soil settled down, may have been performed on each sample about 400 times.

"After seven days' treatment as above, the solutions were filtered; 500 c.c., *i.e.*, the solution from 50 grams of soil, were used for each determination. In each case the solution was evaporated to dryness in a platinum basin, and gently incinerated at a low temperature. The residue was dissolved in pure hydrochloric acid, evaporated to dryness, re-dissolved and filtered. In the filtrate the phosphoric acid or potash, as the case might be, was determined by the methods already described, and as the actual precipitate weighed represented 50 grams of soil, the multiplication of any experimental error in converting into percentages was minimised."

(5) **Organic Carbon.**—The two following modifications of the chromic acid method are recommended; the first by Dr. Mann, the second is used in the Pusa Laboratory:—

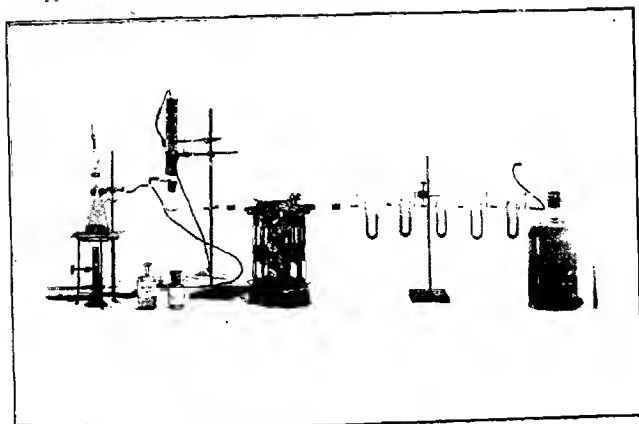
*Dr. Mann's modification.*—Ten grams of the soil passed through a sieve with 1 m.m. round holes are placed in a flask of 200 to 300 c.c. capacity, provided with an india-rubber stopper through which pass two tubes, one for the supply of liquids and the other for the delivery of gas. The gas delivery tube passes first through an inverted condenser about 6 inches long. The tube for supply of liquids is fitted at the upper end with a tube containing moist solid caustic potash. The soil is first treated with 20 c.c. water and 30 c.c. strong sulphuric acid and an aspirator attached to the upper end of the short condenser. The soil with the acid is then heated on the water bath and a slow current of air passed through the apparatus for half an hour, when

all the carbonates are decomposed and the carbonic acid driven off without any serious concentration of the acid.

The upper end of the condenser is now connected with (1) a  $\text{CaCl}_2$  tube, and (2) potash bulbs (followed by  $\text{CaCl}_2$  tube as usual), and 6 grams finely powdered bichromate of potash are added to the flask.  $\text{CO}_2$  is at once evolved, and a gentle current is kept going through the apparatus by means of an aspirator. After the pressure becomes again normal, the water bath is again heated, and kept boiling for five hours, the aspirator being kept going gently all the time. Twenty minutes further are allowed to sweep any  $\text{CO}_2$  remaining out of the apparatus, and then the potash bulbs are weighed.

The carbon calculated from this increase in weight has to be multiplied by 1.25, this being the constant relationship shown by this method to that of dry combustion, and then the organic matter is to be calculated by reckoning it to contain 58 per cent. carbon (multiplied by 1.724).

*Pusa method.*—A suitable quantity of soil is heated carefully in a flask with some potassium bichromate and sulphuric acid plus water. The water is vapourised and expelled from the reaction flask, so that the sulphuric acid gradually becomes more and more concentrated. The steam and other gases pass into a vertical condenser, and the water falls from this into a small flask below. The damp gases pass from the upper end of the condenser to a combustion tube containing red hot





copper oxide, and from this through drying tubes and soda lime tubes as in any determination of carbon dioxide. After the oxidation process is complete in the flask, air is aspirated through the apparatus in the manner customary for combustion or carbon dioxide determinations. The reason why somewhat diluted sulphuric acid is adopted for the initial stages of the oxidation process is because otherwise the sulphuric and chromic acids react together irrespective of the carbonaceous matter present and invariably give a low result; and the reason for distilling the water out of the reaction flask is to admit of bringing concentrated sulphuric acid to bear on the organic matter towards the end of the oxidation. The inclusion of the copper oxide tube is to provide for the oxidation of any carbon monoxide which may be formed. The result of this determination naturally includes the carbon dioxide of the carbonates in the soil. The latter must therefore be estimated separately and deducted.

The subjoined illustration shows the apparatus. The oxidation is carried out in the Erlenmeyer flask, which has a tapped funnel and ground stopper. It is attached to the small spherical flask, and this is attached to the vertical condenser. Then follows the combustion tube, and finally the absorption tubes. Two sulphuric acid drying tubes are inserted, of which the second is weighed. Its weight should remain practically constant until the first requires replacement. Two soda lime tubes should be generally sufficient. The aspirator is only attached at the conclusion of the oxidation. The amount of water which collects in the spherical flask measures about 10 or 15 c.c., and remains constantly hot so that no carbon dioxide is retained. However, it may be boiled if desired.

The time occupied for the oxidation is about half an hour, and one hour may be allowed for the whole analysis.

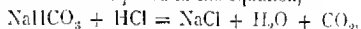
(4) **Carbon Dioxide of Carbonates.**—The methods recommended are:—(a) Generation of  $\text{CO}_2$  in a suitable flask and absorption in U tubes and weighing, (b) Amos's method, as described in *Four. Agri Sci.*, Vol. 1, Part 3, Oct. 1905, namely:—

A weighed quantity of soil is put into the flask B and shaken up with 75 c.c. of carbon dioxide free water (any weight of soil may be used up to 50 grams, containing not more than 0.5 gram of lime); the apparatus is now connected up as in the figure except that the end Y of the rubber tube is connected directly with the pump, and a stream of air is drawn through the apparatus, so that all the atmospheric

carbon dioxide is swept out; the pump is stopped and the Reiset C introduced between Y and the pump, and the pump started again, so that a steady stream of air free from carbon dioxide is drawn through the apparatus. Twenty c.c. of strong hydrochloric acid are now run into B by means of the dropping funnel, and the contents of B gradually brought to the boiling point: the boiling is continued for 20 minutes to ensure all the carbon dioxide being swept into the Reiset C.

The titration is carried out in the lower part of the Reiset apparatus, into which the contents of the absorbing tube are washed, phenolphthalein is added, and normal hydrochloric acid run in until the pink colour begins to fade, then deci-normal hydrochloric acid is run in until the colour is completely discharged, the reading of the deci-normal hydrochloric acid is now taken, methyl-orange is added, and the titration continued until the methyl-orange shows an acid reaction; a second reading of the hydrochloric acid is now taken.

The difference between the two readings gives the volume of deci-normal hydrochloric acid required in the equation,



and hence we get the weight of calcium carbonate originally present in the soil.

The object of employing normal hydrochloric acid in the first part of the titration is to prevent unnecessary dilution, but the liquid must be kept in motion so that the acid is never in excess at any point with consequent evolution of  $\text{CO}_2$ .

(5) **Sulphates.**—The soil should not be ignited before extracting sulphates.

(6) **Potash.**—The Lindo-Gladding method is recommended.

#### Lindo-Gladding Method.

##### (1) *Preparation of Reagents.*

(a) *Ammonium Chloride solution.*—Dissolve 100 grams of ammonium chloride in 500 c.c. of water, add from 5 to 10 grams of pulverized potassium-platinic chloride, and shake at intervals for six or eight hours. The mixture is allowed to settle over night and filtered and the residue is ready for the preparation of a fresh supply.

(b) *Platinum solution.*—The platinum solution used contains 1 gram of metallic platinum (2.1 grams of  $\text{H}_2\text{PtCl}_6$ ) in every 10 c.c.

(2) *Methods of making Solution.*

(a) *With potash salts and mixed fertilizers.*—Boil 10 grams of the sample with 300 c.c. of water (thirty minutes). In the case of mixed fertilizers, add to the hot solution a slight excess of ammonia and then sufficient powdered ammonium oxalate to precipitate all the lime present. Cool, dilute to 500 c.c., mix and pass through a dry filter. In case of muriate and sulphate of potash, sulphate of potash and magnesia and kainit, dissolve and dilute to 500 c.c. without the addition of ammonium and ammonium oxalate.

(b) *With organic compounds.*—When it is desired to determine the total amount of potash in organic substances, such as cotton-seed meal, tobacco stems, etc., saturate 10 grams with strong sulphuric acid, and ignite in a muffle at a low red heat to destroy organic matter. Add a little strong hydrochloric acid, warm slightly in order to loosen the mass from the dish, and proceed as directed under (3) (a).

(3) *Determination.*

(a) *In mixed fertilizers.*—Evaporate 50 c.c. of the solution made according to (2), corresponding to 1 gram of the sample, nearly to dryness, add 1 c.c. of dilute sulphuric acid (1 to 1), evaporate to dryness and ignite to whiteness. As all the potash is in form of sulphate, no loss need be apprehended by volatilization of potash, and a full red heat must be maintained until the residue is perfectly white. Dissolve the residue in hot water, using at least 20 c.c. for each decigram of  $K_2O$ , add a few drops of hydrochloric acid and platinum solution in excess. Evaporate on a water bath to a thick paste and treat the residue with 80 per cent. alcohol, sp. gr. 0.8645, avoiding the absorption of ammonia. Wash the precipitate thoroughly with 80 per cent. alcohol both by decantation and on the filter, continuing the washing after the filtrate is colourless. Wash finally with 10 c.c. of the ammonium chloride solution (1) (a) to remove impurities from the precipitate and repeat this washing five or six times. Wash again thoroughly with 80 per cent. alcohol and dry the precipitate for thirty minutes at  $100^{\circ}$ . The precipitate should be perfectly soluble in water.

(7) *Phosphoric acid.*—Any standard method.

(8) *Nitrates.*—Warrington's modification of Schloesing's method is recommended (*vide Journal of the Chemical Society*, 1882, 345; and Sutton's Volumetric Analysis, page 263.)

**FEEDING STUFFS.**

(1) **Proteids.**—The two methods for the separation of the proteids recommended are precipitation with (a) Stutzer's Copper Hydrate and (b) Mallet's Phospho-tungstic acid.

(a) *Stutzer's Precipitant.*—100 grams of Copper Sulphate are dissolved in 5 litres of water and 2·5 grams of glycerine added. Enough dilute sodium hydrate is added to render the whole slightly alkaline. The precipitated copper hydrate is poured on to a filter, allowed to drain, returned to the bottle and shaken up with water containing 5 grams of glycerine per litre. It is again brought on to a filter and the alternate washing with dilute glycerine and removal of the washing liquid is repeated until the last traces of alkali and sulphate are removed. Finally, the washed copper hydrate is mixed with water containing 10 per cent. glycerine and diluted to one litre. Of this 10 c.c. is usually employed for each determination.

*Method of Precipitating Proteids.*—About one gram of finely ground seeds or cake or two grams of straw are heated to boiling in 100 c.c. of water; then add 5 c.c. of saturated solution of alum and then 10 c.c. of the copper hydrate mixture. Allow to stand for six hours and then filter.

(2) **Starch.**—Horace Brown's modification of O'Sullivan's method (Trans. Guinness Research Laboratories, Vol. I, Part I):—

The details of the new method for determining starch in barley and malt are as follows:—

The grain is first ground very finely in the Maeccker mill and about 5 grams are weighed out into a paper thimble and placed in the extraction apparatus, the moisture being determined on another portion of the ground material.

Ether extraction is omitted, as the fats, besides not being a disturbing element, are equally well extracted by the alcohol.

Eighty c.c. of alcohol of specific gravity 0·920 are introduced into the copper flask, and the working of the apparatus adjusted so that the barley in the thimble is washed with alcohol of sp. gr. 0·900 for three hours,\* when the whole of the reducing substances and alcohol-soluble nitrogen compounds are removed.† It is satisfactory to note that no

\* Malt requires about 9 hours' washing, owing to a higher content of reducing sugars.

† During this alcohol extraction frothing often takes place; to prevent this, it is better, as a rule, to place about 0·5 gram of high melting point paraffin in the copper flask.

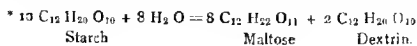
starch can at any time be detected in the extracts, so that loss from this source, which is almost unavoidable when large quantities of water have to be used, is now obviated.

The contents of the thimble are now transferred to a beaker containing about 100 c.c. of water, and the whole thoroughly boiled. After cooling to  $57^{\circ}\text{C}$ ., 10 c.c. of an active malt-extract are added and the conversion is allowed to proceed for 60 minutes. The solution is then boiled, filtered into a 200 c.c. flask, the residue well washed, and the volume adjusted after cooling. The cupric reduction of 20 c.c. of the solution is determined under the standard conditions laid down by Brown, Morris, and Millar (*Journal of the Chemical Society*, 1897, Trans., 94), the maltose being calculated from the copper reduced, according to Table XI in that paper (*loc. cit.*, p. 100), after correction for the reduction due to the malt-extract. The starch equivalent to this maltose is then ascertained, on the assumption, if the conditions of the theoretical equation have been complied with, that 84.4 parts of maltose correspond to 100 parts of starch. If the activity of the malt-extract is not sufficient to convert the starch-products down to this point, due allowance must be made for this.

In this way, the determination of starch in a barley can be carried out in five hours, whilst that of a malt requires from 10 to 11 hours.

The diastatic power of the malt-extract employed is an important consideration in carrying out this method.

In all the experiments so far described in this paper, the malt used had been kiln-dried in the ordinary manner and had a diastatic power of 40 Lintner. Such a malt-extract does not quite give the theoretical equation of page 87\* (the "No. 8 Equation" of Brown and Heron), but providing the lowest limits attainable at  $55^{\circ}$  to  $60^{\circ}\text{C}$ . with such a malt are known, the principle of the method is not affected. For instance, when using a malt with a diastatic capacity of 40 Lintner, it was found by previous experiments on pure starch that the final stage in the reaction was reached when 82.5 per cent. of maltose had been formed from 100 of starch instead of 84.4 parts. It is better, however, to use a malt which gives the theoretical results under the conditions we have laid down, and this, as we have already stated, can be ensured with a malt of a diastatic power of 80 Lintner. If we use malts dried at such a low



temperature as to give a diastatic capacity of 125, or thereabouts, the starch is apt to be over-converted to the extent of 7 to 8 per cent., and irregular results are obtained.

(3) **WOODY FIBRE.**—The material is to be boiled successively for half an hour with (a) 1·25 per cent. caustic soda or potash, (b) 1·25 per cent. of sulphuric acid.

(4) **Acidity.**—The material should be titrated with alkali, using phenol-phthalein as indicator.

(5) **Pentoses.**—The American official method is recommended as detailed on page 25 of Bulletin No. 46, U. S. Department of Agriculture (Division of Chemistry).

*Provisional Method for the Determination of Pentosans by means of Phloroglucin.*

Three grams of the material are placed in a flask, together with 100 c.c. of 12 per cent. hydrochloric acid (specific gravity 1·06) and several pieces of recently heated pumice stone. The flask, placed upon wire gauze, is connected with a condenser and heat applied, rather gently at first, using a gauze top to distribute the flame, and so regulated as to distil over 30 c.c. in about ten minutes. The 30 c.c. driven over are replaced by a like quantity of the dilute acid, and the process continued so long as the distillate gives a pronounced reaction with anilin acetate on filter paper. To the completed distillate is gradually added a quantity of phloroglucin free from diresorcin dissolved in 12 per cent. hydrochloric acid, and the resulting mixture thoroughly stirred. The amount of phloroglucin used should be about double that of the furfural expected. The solution first turns yellow, then green; and very soon an amorphous greenish precipitate appears, which grows rapidly darker, till it finally becomes almost black. The solution is made up to 500 c.c. with 12 per cent. hydrochloric acid, and allowed to stand over night.

The amorphous black precipitate is filtered into a tared gooch through an asbestos felt, washed with 100 c.c. of water, dried to constant weight by heating from three to four hours at 100°, cooled and weighed, the increase in weight being reckoned as phloroglucide. To calculate the furfural from the phloroglucide, use the following formula:—

Phloroglucide (less than and up to 0·2 gram)  $\div 1·82$  = furfural.

Phloroglucide (from 0.2 to 0.3 gram)  $\div 1.895$  = furfural.

Phloroglucide (from 0.3 to 0.4 gram)  $\div 1.92$  = furfural.

Phloroglucide (above 0.4 gram)  $\div 1.93$  = furfural.

To calculate the furfural to pentosan or pentose, use the following formulae:—

I. (furfural 0.0104)  $\times 1.68$  = xylan.

II. (furfural 0.0104)  $\times 2.07$  = araban.

III. (furfural 0.0104)  $\times 1.88$  = pentosan.

IV. (furfural 0.0104)  $\times 1.91$  = xylose.

V. (furfural 0.0104)  $\times 2.35$  = arabinose.

VI. (furfural 0.0104)  $\times 2.13$  = pentose.

*Qualitative test of the purity of the Phloroglucin.*

Dissolve a small quantity of the phloroglucin in a few drops of acetic anhydride, heat almost to boiling, and add a few drops of concentrated sulphuric acid. A violet colour indicates the presence of diresorcin. A phloroglucin which gives more than a faint colouration must be rejected.

(6) **Sugar.**—Soxhlet's modification of Fehling's method:—

*Volumetric determination of reducing Sugars by Fehling's solution.*

(Commercial Organic Analysis, by Allen, Vol. I, page 282.)

The saccharine solution, prepared as already described, and containing from 0.5 to 1.0 gram of sugar per 100 c.c., is placed in a burette. Exactly 10 c.c. of the Fehling's solution are measured into a wide test-tube or small flask supported vertically by a clip. Thirty c.c. of water are added, and a few fragments of tobacco-pipe stem dropped in to prevent bumping. The liquid is heated to boiling by applying a small flame, and the sugar solution run in, 2 c.c. at a time, boiling between each addition. When the blue colour of the liquid has nearly disappeared, the sugar solution should be added more cautiously, but it is desirable to effect the titration as rapidly as possible. The end of the reaction is reached when, on removing the flame and allowing the cuprous oxide to settle, the supernatant fluid appears colourless, or faintly yellow, when viewed against a white surface. If any doubt be felt as to the termination of the reaction, a few drops of the liquid may be filtered through a small filter into a mixture of acetic acid and dilute

potassium ferrocyanide, contained in a porcelain crucible or placed on a white plate. If copper be still present in the liquid, more or less brown colouration will be observed.

The results obtained by using Fehling's solution volumetrically are not generally so accurate as those of the gravimetric method. The operation should be *quickly* conducted.\*

The following are the weights of the principal kinds of sugar which, it is generally assumed, will reduce 10 c.c. of Fehling's solution prepared as described on page 280. Soxhlet's figures are given on page 289.

10 c.c. Fehling solution = '0500 gram of dextrose, levulose or invert sugar.

10 c.c. Fehling solution = '0475 gram of cane sugar (after inversion).

10 c.c. Fehling solution = '0678 gram of milk sugar (lactose).

10 c.c. Fehling solution = '0807 gram of malt sugar (maltose).

In all cases in which Fehling's solution is to be used volumetrically, its true oxidising power under the conditions of the experiment should be ascertained by actual trial. '0475 gram of dry cane sugar, after being inverted as described on page 263, and the solution neutralised, should exactly decolorise 10 c.c. of Fehling's solution.

#### MANURES.

(1) **WATER-SOLUBLE PHOSPHATE.**—The method described in Addyman's *Agricultural Analysis*, page 140, is adopted. It is as follows :—

A portion of the powdery sample is selected as described in the chapter on sampling, Part IV, paragraph 119, and placed in an iron mortar. It is banged with the pestle until a smooth, pasty mass is obtained. This takes two or three minutes. Of course it may happen that a very dry sample has been obtained, in which case it will not become pasty. In such a case it must be

\* In presence of much albuminous or other impurity in the sugar solution, the cuprous oxide refuses to settle, and remains suspended in a fine state of division, rendering the whole liquid muddy. Efficient previous clarification will always prevent this inconvenience and render unnecessary the filtration of a few drops of the turbid liquid, with subsequent testing for copper, by acidulating and adding sulphuretted hydrogen or potassium ferrocyanide.



remembered that the object of triturating in an iron mortar is to break down all hard lumps and to render the mass as homogeneous as possible. When the mass is thoroughly mixed, a portion is taken out with a spatula. It takes some little practice to enable the operator to take off about the right quantity, but if more than three grams or less than one gram has been taken, then another portion should be taken from the mortar. Two grams should be the quantity used. This is weighed out on a watch glass. The next operation is to mix this thoroughly with water. The method which is most effective is somewhat difficult to describe, though in reality very simple. A beaker about  $2\frac{1}{2}$  inches high by  $1\frac{1}{2}$  inch broad is taken, and the weighed mass of 'super' is placed in it by means of a stout glass rod  $3\frac{1}{2}$  inches long. The portion sticking to the watch glass is washed in with not more than 10 c.c. of water. The substance has now to be rubbed round the beaker with the rod very rapidly until it forms with the water a thin paste containing no clots. In doing this the sides of the beaker will become smeared all over with the paste. This is washed to the bottom of the beaker with a jet of cold water, and the liquid made up to about 50 c.c. It is then allowed to stand ten minutes, when it will have settled to a considerable extent. The liquid is decanted through a filter into an 8-oz. beaker. The beaker is filled up again to the same level and decanted off. This is repeated with cold and hot water until all the soluble portion is extracted. The following series of washings should be followed exactly:—

- (a) Stir up with cold water 50 c.c. ;
- (b) Fill up twice with cold water 50 c.c. ;
- (c) Twice with hot water 30 c.c. ;
- (d) Boil smartly with 30 c.c. water.

After the boiling the whole must be transferred to the filter paper and the filtrate tested as it drops from the funnel with blue litmus paper. If an acid reaction is shown, another washing with the hot wash bottle must be given, but as a rule the filtrate is found to be neutral.

(2) **Citrate-Soluble Phosphate.**—The official method of the American Agricultural Chemists as detailed in Bulletin No. 46, U. S.

Department of Agriculture (Division of Chemistry), page 13, is recommended. It is as follows:—

*Citrate-insoluble Phosphoric Acid.*

(a) *In Acidulated Samples.*—Heat 100 c.c. of strictly neutral ammonium citrate solution of 1.09 specific gravity to 65° in a flask placed in a bath of warm water, keeping the flask loosely stoppered to prevent evaporation. When the citrate solution in the flask has reached 65°, drop into it the filter containing the washed residue from the water-soluble phosphoric acid determination, stopper tightly with a smooth rubber, and shake violently until the filter paper is reduced to a pulp. Place the flask in the bath and maintain it at such a temperature that the contents of the flask will stand at exactly 65°. Shake the flask every five minutes. At the expiration of exactly thirty minutes from the time the filter and residue are introduced, remove the flask from the bath and immediately filter the contents as rapidly as possible. Wash thoroughly with water at 65°. Transfer the filter and its contents to a crucible, ignite until all organic matter is destroyed, add from 10 to 15 c.c. of strong hydrochloric acid, and digest until all phosphate is dissolved; or return the filter with contents to the digestion flask, add from 30 to 35 c.c. strong nitric acid, from 5 to 10 c.c. strong hydrochloric acid, and boil until all phosphate is dissolved. Dilute the solution to 200 c.c. If desired, the filter and its contents may be treated according to methods ( $a_2$ ), ( $a_3$ ), or ( $a_4$ ),\* under total phosphoric

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\* Treat 2 grams of the sample by one of the methods given below. After solution, cool, dilute to 200 or 250 c.c., mix, and pour on a dry filter.

( $a_2$ ) Evaporate with 5 c.c. of magnesium nitrate, ignite, and dissolve in hydrochloric acid.

( $a_3$ ) Boil with from 20 to 30 c.c. of strong sulphuric acid, adding from 2 to 4 grams of sodium or potassium nitrate at the beginning of the digestion and a small quantity after the solution has become nearly colourless, or adding the nitrate in small portions from time to time. A Kjeldahl flask marked at 250 c.c. is recommended. After the solution is colourless, add 150 c.c. of water and boil for a few minutes, cool, and make up to mark.

( $a_4$ ) Digest with strong sulphuric acid and such other reagents as are used in either the plain or modified Kjeldahl or Gunning method for estimating nitrogen. Do not add any potassium permanganate, but after the solution has become colourless add about 100 c.c. of water and boil for a few minutes, cool, and make up to a convenient volume; 25 grams of substance and a digestion flask marked at 250 c.c. are recommended.

acid. Mix well; filter through a dry filter; take a definite portion of the filtrate and proceed as under total phosphoric acid.

(*h*) *In Non-Acidulated Samples.*—In case a determination of citrate-insoluble phosphoric acid is required in non-acidulated samples it is to be made by treating two grams of the phosphatic material, without previous washing with water, precisely in the way above described, except that in case the substance contains much animal matter (bone, fish, etc.) the residue insoluble in ammonium citrate is to be treated by one of the processes described under total phosphoric acid ( $a_2$ ), ( $a_3$ ), or ( $a_4$ ).

#### *Citrate-Soluble Phosphoric Acid.*

The sum of the water-soluble and citrate-insoluble subtracted from the total gives the citrate-soluble phosphoric acid.

(3) **Insoluble Phosphate and Total Phosphate.**—Any of the standard methods may be employed.

#### WATERS.

(1) **ALBUMINOID AMMONIA.**—Wanklyn's method :—Use 500 c.c. of the water. After distilling off the free ammonia dilute to the original volume with water, then add 25 c.c. of the alkaline permanganate and distil off 150 c.c., allowing not more than 20 minutes for the distillation.

(2) **Hardness.**—Hehner's method as described in Clowes and Coleman's page 354 is recommended :—

In Hehner's process, the calcium and magnesium carbonates, which cause the temporary hardness, are first determined by titration with standard acid. Both the carbonates and sulphates of calcium and magnesium are then precipitated by adding a known volume of standard sodium carbonate solution in excess and boiling for half an hour (*Note 1*). The unchanged sodium carbonate is then estimated by means of standard acid (*Note 2*). The amount of sodium carbonate which is decomposed by the above sulphates is thus found, and from this the permanent hardness is calculated. The hardness is expressed as parts of  $\text{CaCO}_3$  per 100,000.

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*Note 1.*—If magnesium salts are present, the liquid should be evaporated to dryness after adding the sodium carbonate. The excess of the sodium carbonate is then extracted from the residue by water and is estimated by titration with the standard acid.

*Note 2.*—If this process is used for an alkaline water which contains sodium carbonate, a correction must be applied (page 355).

The following solutions are required :—

(a) *Deci-normal Sulphuric Acid*.—Dilute 100 c.c. of normal sulphuric acid to one litre.

(b) *Deci-normal Sodium Carbonate solution*.—Dissolve 53.06 grams of pure dry sodium carbonate in water, and make this solution up to one litre. The solution may also be prepared by diluting 100 c.c. of normal sodium carbonate solution to a litre.

(c) *Methyl-orange* is used as an indicator in these titrations, because it is not affected by carbonic acid.

**Estimation of the Temporary Hardness.**—Add to 500 c.c. of the water, or less if it is very hard, a few drops of methyl-orange (c). Then add the deci-normal sulphuric acid (a) from a burette, until the colour of the solution just changes to the red tint. From the number of c.c. of acid used, the corresponding amount of  $\text{CaCO}_3$  is calculated, and this is converted into parts per 100,000.

**Estimation of the Permanent Hardness.**—To 250 c.c. of the water add 50 c.c. of the deci-normal sodium carbonate solution (b), and boil for about half an hour. If magnesium salts are present, evaporate to dryness and extract the residue with water. Filter, and wash the precipitate or the insoluble matter with boiled distilled water, and make the cold filtrate up to 250 c.c.

Titrate 50 c.c. of the filtrate with the deci-normal acid (a), using methyl-orange (c) as an indicator.

From the number of c.c. of acid which have been used, the permanent hardness is calculated in terms of  $\text{CaCO}_3$ , and this is converted into parts per 100,000.

**The Total Hardness** may be obtained by adding together the temporary and the permanent hardness.

**Estimation of Sodium Carbonate.**—When the water contains sodium carbonate, it will be distinctly alkaline in reaction, and no permanent hardness will be present. The amount of sodium carbonate present in the water can be easily estimated by means of the standard acid (a), since the sodium carbonate will remain in solution after the water has been boiled. It can therefore be titrated in the filtrate after the carbonates of magnesium and calcium have been precipitated by boiling the water for the determination of the permanent hardness.

**Example.**—In an estimation of temporary hardness in a water, 500 c.c. of the water required 8.4 c.c. of deci-normal sulphuric acid. Now

1 c.c. of this acid corresponds to 0.005 gram of  $\text{CaCO}_3$ ; therefore the weight of  $\text{CaCO}_3$  which would neutralise the acid used in the titration, is  $8.4 \times 0.005 = 0.042$  gram. Hence the parts of calcium carbonate per 100,000 =  $200 \times 0.042 = 8.4$ .

In estimating the permanent hardness, 250 c.c. of the water were boiled with 50 c.c. of deci-normal sodium carbonate solution, and after filtration the filtrate was made up to 250 c.c. Fifty c.c. of this liquid required 8.6 c.c. of deci-normal sulphuric acid for neutralisation.

Hence the permanent hardness in the 50 c.c. used corresponds to  $10 - 8.6 = 1.4$  c.c. of deci-normal sodium carbonate solution, since 10 c.c. of  $\text{Na}_2\text{CO}_3$  solution were present in every 50 c.c. of the solution. Therefore, the number of c.c. of  $\text{Na}_2\text{CO}_3$  solution corresponding to the permanent hardness in the 250 c.c. of the water used =  $1.4 \times 5 = 7$ . And since 1 c.c. of the sodium carbonate solution corresponds to 0.005 gram of  $\text{CaCO}_3$ , the permanent hardness in 100,000 parts =  $7 \times 0.005 \times 400 = 14$ .

Hence the total hardness =  $14 + 8.4 = 22.4$ .

(3) OXYGEN ABSORBED.—The method described by Clowes and Coleman (page 337) was adopted as official. It is as follows:—

The following solutions are required for this process:—

- (a) *Solution of Potassium Permanganate*.—Dissolve 0.395 gram of pure potassium permanganate in 1000 c.c. of water. Each c.c. of this solution contains 0.000395 gram of oxygen available for oxidation.
- (b) *Sodium Thiosulphate Solution*.—Dissolve 1 gram of pure recrystallised sodium thiosulphate in 1 litre of water.
- (c) *Dilute Sulphuric Acid*.—Dilute 1 volume of pure strong sulphuric acid with 3 volumes of water.
- (d) *Potassium Iodide Solution*.—Dissolve 1 part of pure recrystallised potassium iodide in 10 parts of water.
- (e) *Starch Solution*.—Dissolve 1 part of starch in 100 parts of boiling water, and use the clear cold solution.

*The process of estimating the oxygen required by the organic matter.*—Rinse out two 18-oz. conical flasks (A, B), first with strong sulphuric acid to destroy organic matter, and then with distilled water. Measure

into one flask (A) 250 c.c. of pure distilled water, and into the other (B) 250 c.c. of the water under examination. Now measure into each flask 10 c.c. of the standard potassium permanganate solution (a), and 10 c.c. of the dilute sulphuric acid (c), and mix the liquids by shaking them round in the flask. Cover the two flasks with watch glasses, and allow them to stand for three hours at about 15° C. If the pink colour at any time disappears from the water in the flask, the process must be started afresh with 20 c.c. of the permanganate solution.

At the expiration of three hours, add to the liquid in one flask (A) some of the potassium iodide solution (d). The pink colour of the permanganate will change to yellow, since the oxygen of the permanganate liberates its equivalent of iodine. Now add the thiosulphate solution (b) from a burette, until the colour of the liquid in the flask fades to a pale straw tint. At this stage add 1 c.c. of fresh starch solution (e). A deep blue colour will appear: proceed to add more thiosulphate solution gradually until this colour entirely disappears.

Titrate the contents of the other flask (B) in a similar way.

(4) **Nitrates and Nitrites.**—The following are recommended :—

- (a) Copper Zinc couple.
- (b) Picric acid.
- (c) Metaphenylene diamine.



